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AF/1636

Our Reference: UMJ-101-A (UM-1476) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	Peter X. Ma
Serial Number:	09/255,963
Filing Date:	February 23, 1999
Examiner/Art Group Unit:	Sumesh Kaushal, Ph.D./1636
Title:	THREE-DIMENSIONAL HYDROGEL/CELL SYSTEM

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APPEAL BRIEF

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Please enter the following Appeal Brief in the appeal filed October 30, 2003.

REAL PARTY IN INTEREST

The Assignee, the Regents of the University of Michigan, is the real party in interest.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 1-20, 22-23, 25-30, 32-38 and 40-55 are pending and are attached as the Appendix. No claim has been allowed.

STATUS OF AMENDMENTS

An Amendment under Rule 37 C.F.R. § 1.116 was filed on August 28, 2003. In the Advisory Action dated October 24, 2003, the Examiner indicated that the Amendment would be entered for purposes of Appeal.

INTRODUCTION

Appellant submits that an important argument has not been adequately and fully considered by the Examiner during prosecution of the above-identified application. In his discussion of the primary references (Draget et al. and Martinsen et al.) cited in his 35 U.S.C § 103(a) rejection, the Examiner has consistently focused on conditions for initial formation of a hydrogel. However, in contrast, Appellant's invention as defined in claims 1, 11, 23, 34, and 52 recites that the shrinking, swelling or maintaining of the hydrogel (which had already been formed) is selectively controlled. Some examples of the Examiner's irrelevant assertions include the following: "Draget teaches that variation in calcium ion concentration results in the formation of hydrogels with distinct characteristics." Further, the Examiner has consistently asserted that in view of Martinsen, "one would also have been motivated to alter the calcium ion concentration and the ratio of calcium ions to alginate carboxyl groups in order to controlling [sic] the amount of gel swelling and shrinkage." Again, these are conditions for formation of the hydrogel, not for selective control of an already formed hydrogel. Although Martinsen discloses non-homogeneous gel beads in a sodium/calcium ion solution to study how calcium competes with sodium, Martinsen further discloses that when calcium ions saturate the binding sites despite the competition from sodium ions (Fig. 10) "further physical changes are small in the gel system."

In sharp contrast, in Appellant's invention as defined in claims 1, 11, 23, 34, and 52 shrinking, swelling or maintaining of the hydrogel is selectively controlled.

Appellant will show in detail hereinbelow that the Examiner's interpretation of the cited references is not technically or logically correct.

SUMMARY OF THE INVENTION

Every year, millions of Americans suffer tissue loss or end-stage organ failure. Approximately 8 million surgical procedures are performed annually in the United States to treat these disorders. Physicians treat organ or tissue loss by transplanting organs from one individual to another. Although transportation is one of the life-saving therapies, is it seriously limited by donor scarcity. Tissue engineering, which aims at creating biological body parts as alternatives for transplants, offers the possibility of substantial savings by providing substitutes that are less expensive than donor organs and by providing a means of intervention before patients become critically ill (Langer and Vacanti, "Tissue Engineering," Science 260: 920-926 (1993)) (page 1, lines 9-17).

One approach for tissue engineering uses isolated cells or cell substitutions (page 1, lines 21-22). However, isolated cells cannot form new tissues on their own. Most

cells have a requirement for attachment to a surface in order to replicate and function, and require specific environments which often include the presence of supporting material to act as a template for growth (page 1, line 25 – page 2, line 1). Three dimensional scaffolds serve both as a physical support and as an adhesive substrate (U.S. Patent No. 5,514,378 to Mikos et al. [1996]). Natural and synthetic polymers may be used to form highly porous scaffolds (page 2, line 8). Synthetic polymers generally give good reproducibility and controlled release kinetics compared to natural polymers, however, natural polymers are advantageous in that they contain information that facilitates cell attachment or maintenance of differentiated function (Langer and Vacanti, supra) (page 2, lines 19-25). Therefore, what is needed is a method for controlling three-dimensional structures of hydrogel/cell constructs from natural polymers, for use as highly porous scaffolds that permit the support of cells (page 2, lines 26-28).

The present invention provides a novel method for growing cells in a three-dimensional hydrogel/cell system, in particular for growing cells for the fabrication of tissues and organs. In one embodiment of the method, ionically crosslinked alginate gels are used as scaffolds with defined dimensions for *in vitro* engineering applications (page 3, lines 4-6). An embodiment which contemplates a method for tissue engineering *in vitro* includes a) providing an alginate salt, a source of calcium ions, and a calcium releasing agent, b) mixing the alginate salt with the source of calcium ions to provide a mixture, and c) adding the calcium releasing compound to provide a three-dimensional crosslinked hydrogel system (page 3, lines 7-12). Still further, the method may include the step of culturing the three-dimensional crosslinked hydrogel system for growing cells *in vitro* (page 3, lines 12-13). The alginate salt is not limited to a specific type and may be a combination of alginate materials (page 3, lines 18-19). In an alternate embodiment of a method for tissue engineering *in vitro* includes the steps of 1) providing: cells, an alginate salt, a source of calcium ions, and a calcium releasing compound; 2) mixing the cells, alginate salt, and the source of calcium ions to provide a mixture; 3) adding the calcium releasing compound to the mixture to provide a crosslinked gel; and 4) culturing the crosslinked gel to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro* (page 4, line 26 – page 5, line 2). In one embodiment, the source of calcium ions is calcium carbonate and the calcium-releasing compound is D-glucono-δ-lactone. Still further, an embodiment of the method includes the step of implanting the

three-dimensional crosslinked hydrogel system (page 4, lines 13-14). The three-dimensional crosslinked hydrogel system may have any composition (page 4, lines 20-21).

As discussed further below, the three dimensional gel structure with incorporated cells can be maintained in an *in vitro* tissue culture environment by adjusting calcium ion concentration in the culture medium (page 8, lines 3-5).

One of the major concerns in using alginate gels as scaffolds for *in vitro* tissue engineering was the structural instability of the hydrogels in a tissue culture environment. The swelling experiments were designed to understand how the ionically crosslinked alginate gels behave in various aqueous solutions, and to develop ways to control the shape and size of the gels in a tissue culture environment. Sets of three circular gel discs prepared from 3.18% *LH* alginate with 1.5X $CaCO_3$ were immersed in saline (0.9% NaCl aqueous solution) adjusted to varying calcium ion concentrations (Figure 7). The alginate gels swelled when the calcium ion concentration was low (0.0005 and 0.0010 M) while the gels shrank when the calcium ion concentration was high (0.0040 M). At calcium ion concentrations of 0.0020 and 0.0030 M, there was nearly no change in the gel weight over a two-week immersion experiment. Swelling experiments were also conducted with the gels (3.18% *LH* alginate with 1.5X $CaCO_3$) in "complete medium" adjusted to varying calcium concentrations. Again, the gels swelled at low calcium ion concentrations, but shrank at high calcium concentrations (data not shown). At a calcium concentration of 0.0030 M, the gel weight did not change significantly over immersion time. These results clearly showed that the size of ionically crosslinked alginate gels were controlled by the ion concentration of the medium. (Page 15, line 27 – Page 16, line 16)

GROUPING OF CLAIMS

Claims 1-20, 22-23, 25-30, 32-38 and 40-55 are separately patentable from any other claim. Claims 2, 11-20, 33, 34, 41-45 and 55 are separately patentable from any other claim. Claims 48 and 49 are separately patentable from any other claim. Claims 22, 32, 40, 46, 47 and 52 are separately patentable from any other claim. The specific reasons for the separate patentability of each group of claims is set forth in the argument section of this Appeal Brief.

ISSUES ON APPEAL

1. Whether claims 1-20, 22-23, 25-38, and 40-55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89,

1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest the selective size control of a three-dimensional hydrogel system by varying cation concentration of a separate medium into which the hydrogel is introduced?

2. Whether claims 2, 11-20, 33, 34, 41-45 and 55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest culturing the crosslinked hydrogel in a separate medium to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro*?

3. Whether claims 48 and 49 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references teach or suggest the selective size control of a three-dimensional crosslinked hydrogel with a structurally homogeneous composition?

4. Whether claims 22, 32, 40, 46, 47 and 52 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references teaches swelling of a three dimensional hydrogel at 0.0005M – 0.001M, shrinking at 0.004M and maintaining at 0.002M – 0.003M?

ARGUMENT

The Examiner's Rejection

Claims 1-20, 22-23, 25-38, and 40-55 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Draget et al. (Carb. Poly. 14:159-178, 1991), in view of Martinsen et al. (Biotech. Bioeng. 33:79-89, 1989), further in view of Hauselmann et al. (U.S. Patent No. 5,658,343) ('343) and Cao et al. (Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996).

The Examiner stated that Draget et al. teaches the formation of a gel consisting of mixing 15mM $CaCO_3$ with sodium alginate solution, then adding 30mM

GDL, resulting in a final gel of pH 7 only to avoid formation of acidic gels. The Examiner further stated that Draget also teaches that the sodium alginate can be substituted with alginate derived from *Marocystis pyrifera* or *Laminaria hyperbores*, thus altering the viscosity of the gel. Further, the Examiner asserted that the dimensions of the Draget gel are largely a function of the dimensions of the mold into which they form, and can thus be easily modified by one of ordinary skill in the art. (It is submitted that this statement is irrelevant to the issues at hand, as will be shown hereinbelow.) Furthermore, the Examiner stated that Draget teaches that the maximum gel strength was reached when Ca^{2+} concentration was equivalent to the amount of guluronic acid residues and syneresis became prominent when the calcium contents exceeded this value. The Examiner concluded that

> Draget "clearly teaches that variation in calcium ion concentration results in the **formation** of hydrogels with distinct characteristics." (emphasis added)

The Examiner stated that Martinsen teaches that physical properties of beads strongly are dependent upon the composition, sequential structure and molecular size of the polymers. Further, the Examiner stated that the cited art teaches that beads with the highest mechanical strength, lowest shrinkage, best stability towards mono-valent cations and highest porosity were made from alginate with content of L-guluronic acid higher than 70% and average length of G-blocks higher than 15. The Examiner stated that this reference teaches evaluation of stability of Ca-alginate gel beads towards Na+ ions by transferring gel beads to solutions containing different concentrations of $CaCl_2$ and measuring the bead volume (shrinkage) every 24 hours for 3 days. The Examiner further states that Martinsen teaches that gel strength and shrinkage is the function of $CaCl_2$ concentration and gelling time (again, it is submitted that gel shrinkage during formation of the gel is irrelevant to the issues at hand). In addition, the Examiner stated that Martinsen teaches that high gel strength, low shrinkage, high stability towards Na+ ions and high permeability of alginates are the most advantageous factors for the immobilization of living cells.

As such, the Examiner concludes that it would have been obvious in view of Martinsen to control the hydrogel shrinkage or swelling by transferring the hydrogels into solutions that contain different concentrations of calcium ions.

The Examiner further asserted that Hauselmann at al. teaches the method of producing an extracellular matrix, for implantation *in vivo*. The Examiner stated that Hauselmann at al. also teaches that the molar ratio of calcium ions to carboxyl groups in the gel determines the amount of cross-linking of the gel, as well as the amount of swelling (again, this is during formation of the gel, and thus is irrelevant to the issues at hand) and thus size of the gel.

The Examiner stated that Cao et al. teach the method of making and using biodegradable calcium alginate gels with osteoblasts *in vitro* for the implantation *in vivo* to generate bone growth. He also stated that the osteoblasts were suspended in 1% sodium alginate, then 0.2g of calcium sulfate was added to each milliliter of the mixture to initiate gel formation. Then, the Examiner stated that the mixture was injected into nude mice, which resulted in the new bone formation in the transplanted animals.

In conclusion, the Examiner stated that it would have been obvious to the skilled artisan to modify the teaching of Draget and Martinsen by introducing cells (osteoblasts) as taught by Hauselmann and Cao to the calcium-alginate hydrogels' composition. The Examiner further stated that one would have been motivated to utilize the gel as a scaffold for cell growth and differentiation for tissue engineering.

Further, the Examiner asserted that it would have been obvious in view of Martinsen to control the hydrogel shrinkage or swelling by transferring the hydrogels into the solutions that contain different concentration of calcium ions. He states that one would have been motivated to alter the calcium ion concentrations and the ratio of calcium ions to alginate carboxyl groups (again, irrelevant as this ratio corresponds to formation of the gel) in order to control the amount of gel swelling and shrinkage because these characteristics are highly desirable in tissues for different applications. The Examiner further asserted that the Appellant's invention pertaining to specific ion concentrations and molar ratios that results in hydrogel swelling and shrinking are the result of effective variables, which could have been readily determined in view of Draget and Martinsen.

In response to Appellant's arguments in the Amendment filed on August 28, 2003, the Examiner defended his assertions by stating that the Appellant failed to consider the combined teaching of the references, and that the combination and modification of the teachings of the prior art clearly suggested the claimed invention.

ISSUE 1. **Whether claims 1-20, 22-23, 25-38, and 40-55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, in view of Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in further view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest the selective size control of a three-dimensional hydrogel system by varying cation concentration of a separate medium into which it is introduced?**

Appellant's answer: yes

Examiner's answer: no

As highlighted above, the Examiner contends that these references, in combination, teach or suggest control of gel shrinkage and swelling by optimizing calcium ion and sodium ion concentration. **However, contrary to the Examiner's statement, none of the cited references, either alone or in combination, teach or suggest the selective size control of a three dimensional hydrogel system by varying cation concentration of a separate medium into which it is introduced.**

Generally, the references teach the following:

1) Draget et al. teaches that cation concentration in the mixture which forms hydrogels is a parameter to consider during such hydrogel formation;
2) Martinsen teaches that after binding sites at the surface of a gel bead are full, further physical changes in the gel system are small;
3) Hauselmann teaches that mechanical boundary layers may be used to control a hydrogel system; and
4) Cao teaches that a calcium source may be used to form crosslinks during formation of alginate gels.

The Appellant points out that Draget is silent as to transferring a hydrogel into a separate medium. As such, the Examiner's assertion that Draget teaches that variation in calcium ion concentration results in the *formation* of hydrogels with distinct characteristics is off point and is irrelevant to the Appellant's invention. Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites that shrinking, swelling or maintaining of the hydrogel is selectively controlled by varying a calcium ion concentration of a **separate medium** into which the hydrogel is introduced. (emphasis added) According to the court in *In re Napier*, 55 F.3d 610, 613 (Fed. Cir. 1995), "[o]bviousness cannot be established by combining the teachings of the prior art to produce

the claimed invention, absent some teaching, suggestion, or incentive supporting the combination." While Draget does teach a method for forming a hydrogel, he does not mention, teach, suggest, or provide any incentive to transfer that hydrogel into a **separate** medium.

In sharp contrast, Appellant's invention as defined in claims 1, 23 and 52 recites "... a separate medium into which hydrogel system is introduced," while Appellant's claim 11 recites "...a separate medium into which the crosslinked hydrogel system is introduced," and Appellant's claim 34 recites "...a separate culture medium into which the hydrogel system is introduced."

Appellant further points out that the Examiner's mention of the molds Draget used to form the hydrogel as determining gel dimensions is irrelevant because Appellant's invention as defined in claims 11, 23 and 52 recites selective control of "a three-dimensional hydrogel system," the gel having already been formed.

Appellant respectfully submits that Martinsen is an example of a traditional means of creating alginate gel beads. 1) Beads are the only structure which can be formed by Martinsen's method; this is in sharp contrast to Appellant's inventive hydrogel as defined in the pending claims, which may take **any** three dimensional shape. 2) The Martinsen beads are formed by allowing droplets of sodium alginate solution to fall into an aqueous solution of $CaCl_2$; this is in sharp contrast to Appellant's methods as defined in claims 1, 11, 23 and 52. Appellant's hydrogel is **not** formed in a calcium **solution**, rather the Appellant's hydrogel as defined in claim 1, 11, 23 and 52 is formed by mixing an alginate salt and a source of calcium ions and then adding a calcium releasing compound to the mixture. 3) The crosslinking density of the Martinsen-formed beads is NOT uniform– the surface is highly crosslinked, and the interior has a low (if any) crosslinking density (this is known in the art); this is also in sharp contrast to Appellant's inventive hydrogel as defined in the pending claims, which is uniformly crosslinked and is structurally homogeneous. Since Appellant's hydrogels are not formed in calcium solutions, the inventive hydrogels do not have a layer (shell) of surface crosslinking, and are therefore open pored, thus calcium ions can move in and out of the hydrogel.

Further, in Figs. 9 and 10 of Martinsen, the authors were studying how calcium competes with sodium, and how stable the gel beads were. (Figs. 7 and 8 of Martinsen are irrelevant to Appellant's recitation of selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a

medium into which the hydrogel system is introduced–Figs. 7 and 8 speak to the calcium chloride concentration of the solution used for gelation.) This is very different from Appellant's selective control of the size of the hydrogel/hydrogel system. Martinsen at p. 89, Col. 1, states in part that "when calcium ions saturate the binding sites despite the competition from sodium ions (Fig. 10), and after binding has proceeded to a maximum on the time scale (Fig. 12), further physical changes are small in the gel system. (emphasis added)

Appellant submits that Martinsen is NOT describing or suggesting selective control of the size of a hydrogel. In fact, as quoted above, once all the Martinsen binding sites (at the surface) are full, "further physical changes are small in the gel system." Martinsen seems to suggest that once binding sites are full, calcium ions may NOT move out of the system.

However, in sharp contrast, Appellant can selectively cause the inventive hydrogel to swell, shrink or maintain its size by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced.

In summary regarding Martinsen: a) the composition and structure of the Martinsen hydrogel beads is quite different from the three dimensional, uniformly crosslinked and structurally homogeneous hydrogel system of Appellant's invention as defined in the claims; b) Martinsen's aim in Figs. 9 and 10 was to study competition between sodium and calcium ions; and c) Martinsen admits that after surface binding sites are full, further physical changes are small.

Due to the disparity between Martinsen's hydrogel beads and Draget's hydrogel, the skilled artisan would **not** have been taught and/or led to believe by Martinsen that if he placed Draget's hydrogel in Martinsen's 0.9% NaCl solution, that he would be able to selectively control the shrinking, swelling or maintaining of Draget's hydrogel by varying the concentration of $CaCl_2$ in the NaCl solution. In fact, Martinsen was published in 1989, and researchers in tissue engineering have been searching for a means of controlling the size of a formed hydrogel; however, prior to Appellant's invention as defined in the pending claims, the efforts had been unsuccessful. "Failure by others to satisfy a long-felt need or develop a commercially successful product is evidence of unobviousness." *Dow Chem. Co. v. American Cyanamid Co.*, 816 F.2d 617, 623 (Fed. Cir. 1987). This would lead one to believe that Martinsen did NOT teach that which the Examiner is asserting the publication taught.

In furthering the argument regarding the failure by others to satisfy a long-felt need, a brief discussion of the Cao reference is relevant. Cao used calcium sulfate as a source of calcium to form crosslinks in alginate gels. Cao implanted his mixture *in vivo* without characterizing the structural homogeneity or mechanical properties. Although the Draget reference had published before his work, neither Cao (an expert in the field of tissue engineering), nor anyone else in the field, has derived a combination of the tissue engineering approach with Draget's gelation thus far.

Hauselmann et al. ('343) controls the hydrogel system by a **mechanical** means (the boundary layers). The passages referred to by the Examiner (Col. 7, lines 29 et seq.) speaks of molar ratio of calcium ions to carboxyl groups in the gel to determine the amount of **crosslinking** during the formation of the gel. In sharp contrast, the Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites selective control of the size of the hydrogel system by varying cation/calcium ion concentration in a separate medium into which the hydrogel is introduced.

Further, it is respectfully submitted that the Examiner, in stating that a combination of Draget with Martinsen teaches volume control of a non-bead, uniformly crosslinked three-dimensional hydrogel has used impermissible hindsight from Appellant's disclosure in order to **assume** that the Martinsen reference teaches or even suggests that **different** hydrogel structures, formed by **different** methods will react the **same** in calcium ion solutions. It is further submitted that, in doing so, the Examiner has made significant errors in the technical field at hand.

Assuming *arguendo* that the Examiner's assertions regarding Martinsen and Draget are correct, the results of such combination, in addition to the combination itself, should be obvious. As taught in Martinsen's Figure 9, the hydrogel bead swelled at calcium ion concentrations of 0.0030 M. In sharp contrast, the Appellant's hydrogel system in the medium remained **the same size** at calcium ion concentrations of 0.0020 M – 0.0030 M. Further, Martinsen teaches that the hydrogel bead shrank at calcium ion concentrations of 0.03 M and 0.05 M. Again, in sharp contrast, Appellant's hydrogel system in the medium shrank at calcium ion concentrations of **0.0040 M (an order of magnitude different from Martinsen)**.

Comparing the numbers at which Appellant's hydrogel system shrank, maintained and swelled with those shown by Martinsen, the results obtained by the

Appellant are not obvious in view of Draget and Martinsen, rather, Appellant's results are **unexpected**.

The Federal Circuit has spoken to the issue of impermissible hindsight on numerous occasions. In *In re David H. Fine*, 837 F.2d 1071, 1073-74 (Fed. Cir. 1988), the court stated:

> To reach a proper conclusion under § 103, the decisionmaker must step backward in time and into the shoes worn by [a person having ordinary skill in the art] when the invention was unknown and just before it was made. In light of *all* the evidence, the decisionmaker must then determine whether . . . the claimed invention as a whole would have been obvious at *that* time to *that* person. 35 U.S.C. § 103. The answer to that question partakes more of a nature of law than of fact, for it is an ultimate conclusion based on a foundation formed of all the probative facts. (emphasis in original) *Id.* at 1073-74, quoting *Panduit Corp. v. Dennison Mfg. Co.*, 810 F.2d 1561, 1566 (Fed. Cir. 1987).

The assumption made by the Examiner regarding **different** hydrogel structures, formed by **different** methods reacting the **same** in calcium ion solutions is not buttressed by any evidence (in fact, it is rebutted by evidence stated above from Figure 9 of Martinsen). This assertion not only seems to be gleaned from information within the Appellant's disclosure, it also flies in the face of the oft-cited maxim that chemistry is an "unpredictable" art. Further, Appellant points out that the calcium ion concentrations at which his hydrogel system in the medium shrinks, maintains size, and swells are very different from those results given by Martinsen. This difference in results lends credence to Appellant's argument that **different** hydrogel structures, formed by **different** methods, will react **differently** in calcium ion solutions (contrary to the assertion of the Examiner).

Further, the Examiner argued that as long as he takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and as long as he does not include knowledge gleaned only from the Appellant's disclosure, a reconstruction is proper. However, the Examiner "cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to depreciate the claimed invention." *Id.* at 1075. Appellant respectfully submits that the Examiner included knowledge gleaned from the Appellant's disclosure upon making his rejections. Appellant bases this submission upon the evidence herein that indicates that none of the cited references, either alone or in combination, teach or suggest the selective size control of a hydrogel system by varying cation concentration of a separate medium to which the system is introduced.

The Examiner might possibly determine that the skilled artisan might find selectively controlling the skrinking or swelling of a hydrogel system by varying calcium ion concentration of a separate medium into which the hydrogel is introduced "**obvious to try**" in view of the cited references. However, in *In re Fine*, 837 F.2d at 1075, the court stated:

> Because neither Warnick nor Eads, alone or in combination, suggests the claimed invention, the Board erred in affirming the Examiner's conclusion that it would have been obvious to substitute the Warnick nitric acid detector for the Eads sulfur dioxide detector in the Eads system. *ACS Hosp. Sys.*, 732 F.2d at 1575-77. The Eads and Warnick references disclose, at most, that one skilled in the art might find it obvious to try the claimed invention. But whether a particular combination might be "obvious to try" is not a legitimate test of patentability. [references omitted] (emphasis added).

At best, the Draget and Martinsen references in view of the '343 patent and Cao et al. show some components in bits and pieces of Appellant's inventive method as defined in the claims. The relevant art recognizes many components and concepts within its domain. Upon close investigation and scrutiny of the diverse practices in this art and its peripheral technical fields of endeavor, a fact finder is inevitably led to the conclusion that artisans can and could produce a myriad of methods and devices of apparently endless diversity from components and concepts already individually recognized as belonging to the prior art. Such speculation must not cloud the standards for evaluation of patentability over the prior art under 35 U.S.C. § 103. Properly focused, the issue centers on what would have been obvious to one of ordinary skill in the art at the time of the invention.

Obviousness is tested by what the combined references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 425 (CCPA 1981). Approaches to obviousness determinations which focus merely on identifying and tabulating missing elements in hindsight retrospect imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge (ie. the selective size control of a three dimensional hydrogel system by varying cation concentration of a separate medium into which it is introduced), and fall victim to the insidious effect of the hindsight syndrome wherein that which only the inventor taught is used against its teacher. (emphasis added). *W.L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1553 (Fed. Cir. 1983).

For all of the above reasons, it is submitted that Appellant's invention as defined in claims 1-20, 22-23, 25-38, and 40-55 is not anticipated, taught, or rendered obvious by Draget et al. in view of Martinsen et al., and further in view of Hauselmann et al. ('343) and Cao et al., either alone or in combination, and patentably defines over the art of record.

As such, it is submitted that the cited references neither teach nor suggest selective control of a hydrogel system by varying cation concentration of a separate medium into which the hydrogel is introduced as defined in Appellant's claims 1, 11, 23, 34 and 52.

ISSUE 2. **Whether claims 2, 11-20, 33, 34, 41-45 and 55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest culturing the crosslinked hydrogel in a medium to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro*?**

Appellant's answer: yes

Examiner's answer: no

It is further submitted that the arguments for patentability of the claims hereinabove and hereinbelow are intended to be cumulative.

As highlighted above, the Examiner contends that it would have been obvious to modify the teachings of Draget and Martinsen by introducing the cells (as taught by Hauselmann and Cao) into a calcium-alginate hydrogel composition. However, as argued above, none of the references teach or suggest culturing the hydrogel in a **separate medium** to provide a hydrogel/cell system for growing cells *in vitro*.

Cao used $CaSO_4$ as a calcium source to form crosslinks in alginate gels. The method of Cao is presented in the Appellant's specification as a control method, which results in poor gel formation (irregular shape and heterogeneity) and inferior mechanical properties. Cao implanted his mixture *in vivo* without characterizing the structural homogeneity or mechanical properties. As such, Cao does not teach or suggest any medium at all.

In sharp contrast, Appellant has found that the mechanical properties of the hydrogel maybe controlled by varying the calcium concentration of a separate medium into which the hydrogel is introduced. Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 further recites that the inventive hydrogel system may be used for growing cells when the hydrogel system in the medium is cultured.

Hauselmann discusses the use of alginate as a suitable matrix to contain and support dispersed cells, but does not mention transfer into a medium. In sharp contrast, the Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 recites culturing the three-dimensional hydrogel system in a **separate medium** for supporting and growing cells.

As discussed in reference to Issue 1, Draget neither teaches nor suggests introducing his hydrogel into any separate medium, let alone a separate medium that is adapted to stabilize the hydrogel to support cell viability.

The Examiner points out that Martinsen states, "...the high gel strength, low shrinkage, high stability towards sodium ions, and high permeability of alginates with high content of guluronic acid will normally be most advantageous for immobilization of living cells." The Appellant fails to see the relevance of this passage to his disclosed invention. Martinsen briefly mentions what "will normally be most advantageous for immobilization of living cells," but fails to teach or suggest a homogeneous crosslinked hydrogel system capable of being selectively controlled by variation of cation concentration of a medium into which the hydrogel is introduced. Martinsen does not disclose a medium at all, but rather simply a $NaCl/CaCl_2$ solution to study competition between sodium and calcium cations.

Due to the disparity between Martinsen's hydrogel beads and Draget's hydrogel, the skilled artisan would **not** have been taught and/or led to believe by Martinsen that if he placed Draget's hydrogel in Martinsen's 0.9% NaCl solution, that he would be able to selectively control the shrinking, swelling or maintaining of Draget's hydrogel by varying the concentration of the $CaCl_2$, as well as maintaining living cells in a medium.

In sharp contrast, Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 recites introduction of cells supported in a medium. Appellant's three dimensional gel structure with incorporated cells can be maintained in an *in vitro* tissue culture environment by adjusting calcium ion concentration in the culture medium.

In *In re David H. Fine*, 837 F.2d at 1073-74, the Federal Circuit stated:

> To reach a proper conclusion under § 103, the decisionmaker must step backward in time and into the shoes worn by [a person having ordinary skill in the art] when the invention was unknown and just before it was made. In light of *all* the evidence, the decisionmaker must then determine whether . . . the claimed invention as a whole would have been obvious at *that* time to *that* person. 35 U.S.C. § 103.

Appellant again submits that the Examiner has engaged in impermissible hindsight in combining the cells of Cao and Hauselmann with the hydrogels of Draget and Martinsen. AS pointed out above, none of the references teach or suggest placing hydrogels in a medium, much less a culture medium as defined in claims 2, 11, 33, 34, 45 and 55. Just as the combination of Draget and Martinsen would NOT have been obvious to one skilled in the art at the time the Appellant's invention was made, neither would a combination of Cao's cells with Draget's homogeneous gelation have been obvious. And even assuming *arguendo* such a combination were made, it would not render selective control of a hydrogel in a medium.

In sharp contrast, Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 recites that the selectively controlled hydrogel system may be cultured in a medium for growing cells. Although the Draget reference had published before his work, neither Cao (an expert in the field of tissue engineering), nor anyone else in the field, has derived a combination of the tissue engineering approach with Draget's homogeneous gelation. This failure to derive such a combination, in addition to the lack of teaching or suggestion by the cited references, indicates that it would NOT "have been obvious at *that* time to *that* person" to prepare and form a "structurally homogeneous and mechanically strong alginate gel with defined dimensions, which can be used to incorporate living cells."

For all of the above reasons, it is submitted that Appellant's invention as defined in claims 2, 11-20, 33, 34, 41-45 and 55 is not anticipated, taught, or rendered obvious by Draget et al. in view of Martinsen et al., and further in view of Hauselmann et al. ('343) and Cao et al., either alone or in combination, and patentably defines over the art of record.

ISSUE 3: Whether claims 48 and 49 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references

teach or suggest a selectively controlled three-dimensional crosslinked hydrogel with a structurally homogeneous composition?

Appellant's answer: yes

Examiner's answer: no

It is further submitted that the arguments for patentability of the claims hereinabove and hereinbelow are intended to be cumulative.

As previously stated, the Examiner argues that it would have been obvious in view of Martinsen to control the hydrogel shrinkage or swelling by transferring the hydrogels into solutions that contain different concentrations of calcium ions. Further, the Examiner argues that it would have been obvious to combine Draget's hydrogel with Martinsen's alleged teaching.

Draget does mention a "homogeneous gel." However, as explained in reference to Issue 1, Draget fails to teach or suggest the introduction of the hydrogel into a separate medium. Therefore, Appellant submits that by combining the Draget homogeneous hydrogel with the alleged teaching of Martinsen, the Examiner is concluding that the homogeneous hydrogel of Draget will behave the same as the non-homogeneous, highly crosslinked surface hydrogel bead of Martinsen.

It is respectfully submitted that the Examiner, in stating that Martinsen teaches volume control of a non-bead, uniformly crosslinked hydrogel has used impermissible hindsight from Appellant's disclosure in order to **assume** that the Martinsen reference teaches or even suggests that **different** hydrogel structures, formed by **different** methods will react the **same** in calcium ion solutions. It is further submitted that, in doing so, the Examiner has made errors in the technical field at hand.

The assumption made by the Examiner regarding **different** hydrogel structures (homogeneous versus highly crosslinked surfaces with low to no crosslinked interior), formed by **different** methods reacting the **same** in calcium ion solutions is not buttressed by any evidence. In fact, as shown above, the Examiner's assertion is rebutted by teachings of Martinsen (Figure 9). The Examiner's assertion not only seems to be gleaned from information within the Appellant's disclosure, it also flies in the face of the oft-cited maxim that chemistry is an "unpredictable" art.

For all of the above reasons, it is submitted that Appellant's invention as defined in claims 48 and 49 is not anticipated, taught, or rendered obvious by Draget et al.

in view of Martinsen et al., and further in view of Hauselmann et al. ('343) and Cao et al., either alone or in combination, and patentably defines over the art of record.

ISSUE 4: Whether claims 22, 32, 40, 46, 47 and 52 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references teaches swelling of a three dimensional hydrogel at 0.0005M – 0.001M, shrinking at 0.004M and maintaining at 0.002M – 0.003M?

Appellant's answer: Yes

Examiner's answer: No

It is further submitted that the arguments for patentability of the claims hereinabove are intended to be cumulative.

The Examiner asserts that the combination of the teachings of Draget and Martinsen is obvious and that the combination in further view of Hauselmann and Cao results in the Appellant's claimed invention. However, Appellant points out that if such a combination is obvious, the results of such combination should also be obvious. In *In re Payne*, 606 F.2d 303, 313 (C.C.P.A. 1979), the court stated, "[a]n obviousness rejection based on similarity in chemical structure and function entails the motivation of one skilled in the art to make a claimed compound, in the expectation that compounds similar in structure will have similar properties." In sharp contrast, the hydrogel of Draget is NOT similar in structure to the hydrogel bead of Martinsen. Further, Appellant submits that even assuming *arguendo* that the skilled artisan combined Draget and Martinsen as suggested by the Examiner, the results of such combination are not obvious.

In Figure 9 of Martinsen, the hydrogel bead swelled at a calcium ion concentration of 0.0030 M. In sharp contrast, the Appellant's hydrogel system in the medium remained the same size at calcium ion concentrations ranging between 0.0020 M – 0.0030 M.

Further, Martinsen teaches that the hydrogel bead shrank at calcium ion concentrations of 0.03 M and 0.05 M. Again, in sharp contrast, Appellant's hydrogel system in the medium shrank at calcium ion concentrations of 0.0040 M. The shrinking of

the Appellant's hydrogel resulted at a calcium ion concentration which is an order of magnitude less than that shown in Martinsen.

Still further, Martinsen teaches the swelling of the hydrogel bead at 0.001 M, 0.003 M and at 0.005 M, the swelling trend generally moving from 0.001M upward. Appellant showed swelling at calcium ion concentrations ranging between about 0.0005 M and about 0.001 M, the swelling trend generally moving from 0.001M downward.

Appellant's results, which are unexpectedly different than those taught by Martinsen, further support the Appellant's assertion that **different** hydrogel structures, formed by **different** methods, will react **differently** in calcium ion solutions (contrary to the assertion of the Examiner).

Appellant respectfully submits that none of the references cited by the Examiner teach or suggest "compounds similar in structure" that "have similar properties" as those of the Appellant's invention as defined in claims 22, 32, 40, 46, 47 and 52.

In summary,

1) Draget et al. teaches that cation concentration in the mixture which forms hydrogels is a parameter to consider during such hydrogel formation. Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites the introduction of an already formed hydrogel into a **separate** medium and selectively controlling shrinking, swelling, and maintaining by varying the cation concentration of a separate medium into which the hydrogel is introduced.
2) Martinsen teaches the formation of gel beads only by allowing droplets of sodium alginate solution to fall into an aqueous solution of $CaCl_2$. Appellant's hydrogel as defined in claim 1, 11, 23, 34 and 52 is **not** formed in a calcium **solution** and can take on any three-dimensional shape.
3) Martinsen teaches a hydrogel with a highly crosslinked surface. After binding sites at the surface of the gel bead are full, further physical changes in the gel system are small. Appellant's inventive hydrogels as defined in claim 1, 11, 23, 34 and 52 do not have a layer (shell) of surface crosslinking, and are therefore open pored, thus calcium ions can move in and out of the hydrogel, allowing selective shrinking, swelling, or maintaining of its size.
4) Hauselmann teaches that mechanical boundary layers may be used to control a hydrogel system. Appellant's invention as defined in claims 1, 11, 23, 34 and 52

recites that selective control of shrinking, swelling, or maintaining the hydrogel is accomplished by varying the calcium ion concentration of a separate medium into which the hydrogel is introduced (no mechanical boundaries).

5) Cao teaches that a calcium source may be used to form crosslinks during formation of alginate gels for direct implantation *in vivo*. Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites culturing the hydrogel in a medium to provide a hydrogel/cell system for growing cells *in vitro*.

CONCLUSION

For the reasons stated above, it is submitted that there is no teaching or suggestion in any of the cited references to selectively control the size of a three-dimensional hydrogel system by varying cation concentration of a separate medium into which the hydrogel is introduced; nor do the cited references teach or suggest culturing the crosslinked hydrogel in a medium to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro*; still further, the cited references do not teach or suggest selective control of a hydrogel with a structurally homogeneous composition; yet further, none of the cited references teach or suggest shrinking, swelling or maintaining of a hydrogel with calcium ion concentration molarities as defined in Appellant's claims.

Thus, it is respectfully submitted that Appellant's invention as set forth in claims 1-20, 22-23, 25-38, and 40-55 patentably defines over the cited references and is not anticipated, taught or rendered obvious thereby.

As such, it is respectfully submitted that the Examiner's final rejection of claims 1-20, 22-23, 25-38, and 40-55 is erroneously based, and its reversal is respectfully requested.

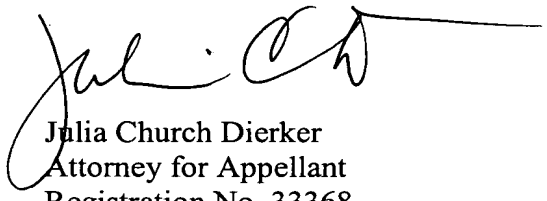
No oral hearing is requested.

Appellant's Attorney's Deposit Account may be charged in the amount of $165.00 to cover the Appeal Brief filing fee.

This Appeal Brief is being filed in triplicate, including one original and two copies.

Respectfully submitted,

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Dated: December 30, 2003
JCD/JRO/jro

APPENDIX
CLAIMS AT ISSUE IN APPEAL OF S.N. 09/255,963

1. A method comprising the steps of:

mixing an alginate salt and a source of calcium ions to provide a mixture;

adding a calcium releasing compound to the mixture to provide a three-dimensional crosslinked hydrogel system; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced.

2. The method of Claim 1, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells in vitro.

3. The method of Claim 1, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

4. The method of Claim 1, wherein the alginate salt is prepared from an alginate source selected from Macrocystis pyrifera and Laminaria hyperborea.

5. The method of Claim 1, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

6. The method of Claim 1, wherein the calcium releasing compound is D-glucono-δ-lactone.

7. The method of Claim 1, wherein the source of calcium ions is calcium carbonate and the calcium releasing compound is D-glucono-δ-lactone, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

8. The method of Claim 1, further comprising the step of implanting the three-dimensional crosslinked hydrogel system.

9. The method of Claim 1, wherein the three-dimensional crosslinked hydrogel system has a thickness of between about 4 mm and about 8 mm, and a diameter of approximately 18 mm.

10. The method of Claim 1, wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio of 0.27.

11. A method for tissue engineering *in vitro*, the method comprising the steps of:

mixing cells, an alginate salt and a source of calcium ions to provide a mixture;

adding a calcium releasing compound to the mixture to provide a crosslinked hydrogel;

selectively controlling shrinking, swelling or maintaining of the crosslinked hydrogel by varying a calcium ion concentration of a separate medium into which the crosslinked hydrogel is introduced; and

culturing the crosslinked hydrogel in the medium to provide a three-dimensional crosslinked hydrogel/cell system for growing the cells *in vitro*.

12. The method of Claim 11, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

13. The method of Claim 11, wherein the alginate salt is prepared from an alginate source selected from Macrocystis pyrifera and Laminaria hyperborea.

14. The method of Claim 11, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

15. The method of Claim 11, wherein the calcium releasing compound is D-glucono-δ-lactone.

16. The method of Claim 11, wherein the source of calcium ions is calcium carbonate and the calcium releasing compound is D-glucono-δ-lactone, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

17. The method of Claim 11, further comprising the step of implanting the three-dimensional crosslinked hydrogel/cell system.

18. The method of Claim 11, wherein the three-dimensional crosslinked hydrogel/cell system has a thickness of between about 4 mm and about 8 mm, and a diameter of approximately 18 mm.

19. The method of Claim 11, wherein the three-dimensional crosslinked hydrogel/cell system has a calcium ion to carboxyl molar ratio of 0.27.

20. The method of Claim 11, wherein the cells are osteoblasts.

22. The method as defined in claim 1 wherein the hydrogel system swelled at calcium ion concentrations in the medium between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration in the medium of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations in the medium between about 0.0020 M and about 0.0030 M.

23. A method for preparing a three-dimensional hydrogel system, the method comprising the steps of:

adding a calcium-releasing compound to a mixture of at least one hydrophilic polymer comprising an alginate salt and a source of calcium cations to provide a three-dimensional crosslinked hydrogel system; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced.

25. The method as defined in claim 23 wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

26. The method as defined in claim 23, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

27. The method as defined in claim 26 wherein the calcium releasing compound is D-glucono-δ-lactone.

28. The method as defined in claim 27 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

29. The method as defined in claim 23 wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

30. The method as defined in claim 29 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

32. The method as defined in claim 23 wherein the hydrogel system swelled at calcium ion concentrations between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations between about 0.0020 M and about 0.0030 M.

33. The method as defined in claim 23, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells in vitro.

34. A three-dimensional crosslinked hydrogel composition, consisting essentially of:

at least one hydrophilic polymer comprising an alginate salt;

a source of calcium cations;

a calcium-releasing compound, whereby a mixture of the at least one hydrophilic polymer, the source of calcium cations and the calcium-releasing compound forms the crosslinked hydrogel composition; and

a separate culture medium into which the hydrogel composition is introduced, the culture medium having a predetermined calcium ion concentration, wherein the predetermined calcium ion concentration determines the shrinking, swelling or maintaining of the crosslinked hydrogel composition.

35. The composition as defined in claim 34, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate; wherein the source of calcium cations is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate; and wherein the calcium-releasing compound is D-glucono-δ-lactone.

36. The composition as defined in claim 35 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

37. The composition as defined in claim 35 wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

38. The composition as defined in claim 37 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

40. The composition as defined in claim 34 wherein when the predetermined calcium ion concentration is between about 0.0020 M and about 0.0030 M, the hydrogel composition remains substantially the same size.

41. The composition as defined in claim 45 wherein the cells are at least one of osteoblasts and cells which secrete a medically useful compound.

42. The method of claim 2 wherein the cells secrete a medically useful compound.

43. The method of claim 11 wherein the cells secrete a medically useful compound.

44. The method of claim 33 wherein the cells are at least one of osteoblasts and cells which secrete a medically useful compound.

45. The three-dimensional crosslinked hydrogel composition as defined in claim 34, further comprising cells incorporated into the hydrogel composition, thereby forming a hydrogel/cell system.

46. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein when the predetermined calcium ion concentration is between about 0.0005 M and about 0.0010 M, the hydrogel composition swelled.

47. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein when the predetermined calcium ion concentration is about 0.0040 M, the hydrogel composition shrank.

48. The method as defined in claim 1 wherein the three-dimensional crosslinked hydrogel system is structurally homogeneous.

49. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein the composition is structurally homogeneous.

50. The method as defined in claim 1 wherein the source of calcium ions is in powder form.

51. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein the source of calcium cations is in powder form.

52. A method for preparing a three-dimensional hydrogel system, the method comprising the steps of:

adding a calcium-releasing compound to a mixture of at least one hydrophilic polymer comprising an alginate salt and a source of calcium cations to provide a three-dimensional crosslinked hydrogel system, wherein the calcium releasing compound is D-glucono-δ-lactone, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate, and wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced, wherein the hydrogel system swelled at calcium ion concentrations between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations between about 0.0020 M and about 0.0030 M;

wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

53. The method as defined in claim 52 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

54. The method as defined in claim 53 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

55. The method as defined in claim 54, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells *in vitro*.

COPY

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Our Reference: UMJ-101-A (UM-1476) PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	Peter X. Ma
Serial Number:	09/255,963
Filing Date:	February 23, 1999
Examiner/Art Group Unit:	Sumesh Kaushal, Ph.D./1636
Title:	THREE-DIMENSIONAL HYDROGEL/CELL SYSTEM

APPEAL BRIEF

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Please enter the following Appeal Brief in the appeal filed October 30, 2003.

REAL PARTY IN INTEREST

The Assignee, the Regents of the University of Michigan, is the real party in interest.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 1-20, 22-23, 25-30, 32-38 and 40-55 are pending and are attached as the Appendix. No claim has been allowed.

STATUS OF AMENDMENTS

An Amendment under Rule 37 C.F.R. § 1.116 was filed on August 28, 2003. In the Advisory Action dated October 24, 2003, the Examiner indicated that the Amendment would be entered for purposes of Appeal.

INTRODUCTION

Appellant submits that an important argument has not been adequately and fully considered by the Examiner during prosecution of the above-identified application. In his discussion of the primary references (Draget et al. and Martinsen et al.) cited in his 35 U.S.C § 103(a) rejection, the Examiner has consistently focused on conditions for initial formation of a hydrogel. However, in contrast, Appellant's invention as defined in claims 1, 11, 23, 34, and 52 recites that the shrinking, swelling or maintaining of the hydrogel (which had already been formed) is selectively controlled. Some examples of the Examiner's irrelevant assertions include the following: "Draget teaches that variation in calcium ion concentration results in the formation of hydrogels with distinct characteristics." Further, the Examiner has consistently asserted that in view of Martinsen, "one would also have been motivated to alter the calcium ion concentration and the ratio of calcium ions to alginate carboxyl groups in order to controlling [sic] the amount of gel swelling and shrinkage." Again, these are conditions for formation of the hydrogel, not for selective control of an already formed hydrogel. Although Martinsen discloses non-homogeneous gel beads in a sodium/calcium ion solution to study how calcium competes with sodium, Martinsen further discloses that when calcium ions saturate the binding sites despite the competition from sodium ions (Fig. 10) "further physical changes are small in the gel system."

In sharp contrast, in Appellant's invention as defined in claims 1, 11, 23, 34, and 52 shrinking, swelling or maintaining of the hydrogel is selectively controlled.

Appellant will show in detail hereinbelow that the Examiner's interpretation of the cited references is not technically or logically correct.

SUMMARY OF THE INVENTION

Every year, millions of Americans suffer tissue loss or end-stage organ failure. Approximately 8 million surgical procedures are performed annually in the United States to treat these disorders. Physicians treat organ or tissue loss by transplanting organs from one individual to another. Although transportation is one of the life-saving therapies, is it seriously limited by donor scarcity. Tissue engineering, which aims at creating biological body parts as alternatives for transplants, offers the possibility of substantial savings by providing substitutes that are less expensive than donor organs and by providing a means of intervention before patients become critically ill (Langer and Vacanti, "Tissue Engineering," Science 260: 920-926 (1993)) (page 1, lines 9-17).

One approach for tissue engineering uses isolated cells or cell substitutions (page 1, lines 21-22). However, isolated cells cannot form new tissues on their own. Most

cells have a requirement for attachment to a surface in order to replicate and function, and require specific environments which often include the presence of supporting material to act as a template for growth (page 1, line 25 – page 2, line 1). Three dimensional scaffolds serve both as a physical support and as an adhesive substrate (U.S. Patent No. 5,514,378 to Mikos et al. [1996]). Natural and synthetic polymers may be used to form highly porous scaffolds (page 2, line 8). Synthetic polymers generally give good reproducibility and controlled release kinetics compared to natural polymers, however, natural polymers are advantageous in that they contain information that facilitates cell attachment or maintenance of differentiated function (Langer and Vacanti, supra) (page 2, lines 19-25). Therefore, what is needed is a method for controlling three-dimensional structures of hydrogel/cell constructs from natural polymers, for use as highly porous scaffolds that permit the support of cells (page 2, lines 26-28).

The present invention provides a novel method for growing cells in a three-dimensional hydrogel/cell system, in particular for growing cells for the fabrication of tissues and organs. In one embodiment of the method, ionically crosslinked alginate gels are used as scaffolds with defined dimensions for *in vitro* engineering applications (page 3, lines 4-6). An embodiment which contemplates a method for tissue engineering *in vitro* includes a) providing an alginate salt, a source of calcium ions, and a calcium releasing agent, b) mixing the alginate salt with the source of calcium ions to provide a mixture, and c) adding the calcium releasing compound to provide a three-dimensional crosslinked hydrogel system (page 3, lines 7-12). Still further, the method may include the step of culturing the three-dimensional crosslinked hydrogel system for growing cells *in vitro* (page 3, lines 12-13). The alginate salt is not limited to a specific type and may be a combination of alginate materials (page 3, lines 18-19). In an alternate embodiment of a method for tissue engineering *in vitro* includes the steps of 1) providing: cells, an alginate salt, a source of calcium ions, and a calcium releasing compound; 2) mixing the cells, alginate salt, and the source of calcium ions to provide a mixture; 3) adding the calcium releasing compound to the mixture to provide a crosslinked gel; and 4) culturing the crosslinked gel to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro* (page 4, line 26 – page 5, line 2). In one embodiment, the source of calcium ions is calcium carbonate and the calcium-releasing compound is D-glucono-δ-lactone. Still further, an embodiment of the method includes the step of implanting the

three-dimensional crosslinked hydrogel system (page 4, lines 13-14). The three-dimensional crosslinked hydrogel system may have any composition (page 4, lines 20-21).

As discussed further below, the three dimensional gel structure with incorporated cells can be maintained in an *in vitro* tissue culture environment by adjusting calcium ion concentration in the culture medium (page 8, lines 3-5).

One of the major concerns in using alginate gels as scaffolds for *in vitro* tissue engineering was the structural instability of the hydrogels in a tissue culture environment. The swelling experiments were designed to understand how the ionically crosslinked alginate gels behave in various aqueous solutions, and to develop ways to control the shape and size of the gels in a tissue culture environment. Sets of three circular gel discs prepared from 3.18% *LH* alginate with 1.5X $CaCO_3$ were immersed in saline (0.9% NaCl aqueous solution) adjusted to varying calcium ion concentrations (Figure 7). The alginate gels swelled when the calcium ion concentration was low (0.0005 and 0.0010 M) while the gels shrank when the calcium ion concentration was high (0.0040 M). At calcium ion concentrations of 0.0020 and 0.0030 M, there was nearly no change in the gel weight over a two-week immersion experiment. Swelling experiments were also conducted with the gels (3.18% *LH* alginate with 1.5X $CaCO_3$) in "complete medium" adjusted to varying calcium concentrations. Again, the gels swelled at low calcium ion concentrations, but shrank at high calcium concentrations (data not shown). At a calcium concentration of 0.0030 M, the gel weight did not change significantly over immersion time. These results clearly showed that the size of ionically crosslinked alginate gels were controlled by the ion concentration of the medium. (Page 15, line 27 – Page 16, line 16)

GROUPING OF CLAIMS

Claims 1-20, 22-23, 25-30, 32-38 and 40-55 are separately patentable from any other claim. Claims 2, 11-20, 33, 34, 41-45 and 55 are separately patentable from any other claim. Claims 48 and 49 are separately patentable from any other claim. Claims 22, 32, 40, 46, 47 and 52 are separately patentable from any other claim. The specific reasons for the separate patentability of each group of claims is set forth in the argument section of this Appeal Brief.

ISSUES ON APPEAL

1. Whether claims 1-20, 22-23, 25-38, and 40-55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89,

1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest the selective size control of a three-dimensional hydrogel system by varying cation concentration of a separate medium into which the hydrogel is introduced?

2. Whether claims 2, 11-20, 33, 34, 41-45 and 55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest culturing the crosslinked hydrogel in a separate medium to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro*?

3. Whether claims 48 and 49 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references teach or suggest the selective size control of a three-dimensional crosslinked hydrogel with a structurally homogeneous composition?

4. Whether claims 22, 32, 40, 46, 47 and 52 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references teaches swelling of a three dimensional hydrogel at 0.0005M – 0.001M, shrinking at 0.004M and maintaining at 0.002M – 0.003M?

ARGUMENT

The Examiner's Rejection

Claims 1-20, 22-23, 25-38, and 40-55 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Draget et al. (Carb. Poly. 14:159-178, 1991), in view of Martinsen et al. (Biotech. Bioeng. 33:79-89, 1989), further in view of Hauselmann et al. (U.S. Patent No. 5,658,343) ('343) and Cao et al. (Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996).

The Examiner stated that Draget et al. teaches the formation of a gel consisting of mixing 15mM $CaCO_3$ with sodium alginate solution, then adding 30mM

GDL, resulting in a final gel of pH 7 only to avoid formation of acidic gels. The Examiner further stated that Draget also teaches that the sodium alginate can be substituted with alginate derived from *Marocystis pyrifera* or *Laminaria hyperbores*, thus altering the viscosity of the gel. Further, the Examiner asserted that the dimensions of the Draget gel are largely a function of the dimensions of the mold into which they form, and can thus be easily modified by one of ordinary skill in the art. (It is submitted that this statement is irrelevant to the issues at hand, as will be shown hereinbelow.) Furthermore, the Examiner stated that Draget teaches that the maximum gel strength was reached when Ca^{2+} concentration was equivalent to the amount of guluronic acid residues and syneresis became prominent when the calcium contents exceeded this value. The Examiner concluded that

> Draget "clearly teaches that variation in calcium ion concentration results in the **formation** of hydrogels with distinct characteristics." (emphasis added)

The Examiner stated that Martinsen teaches that physical properties of beads strongly are dependent upon the composition, sequential structure and molecular size of the polymers. Further, the Examiner stated that the cited art teaches that beads with the highest mechanical strength, lowest shrinkage, best stability towards mono-valent cations and highest porosity were made from alginate with content of L-guluronic acid higher than 70% and average length of G-blocks higher than 15. The Examiner stated that this reference teaches evaluation of stability of Ca-alginate gel beads towards Na+ ions by transferring gel beads to solutions containing different concentrations of $CaCl_2$ and measuring the bead volume (shrinkage) every 24 hours for 3 days. The Examiner further states that Martinsen teaches that gel strength and shrinkage is the function of $CaCl_2$ concentration and gelling time (again, it is submitted that gel shrinkage during formation of the gel is irrelevant to the issues at hand). In addition, the Examiner stated that Martinsen teaches that high gel strength, low shrinkage, high stability towards Na+ ions and high permeability of alginates are the most advantageous factors for the immobilization of living cells.

As such, the Examiner concludes that it would have been obvious in view of Martinsen to control the hydrogel shrinkage or swelling by transferring the hydrogels into solutions that contain different concentrations of calcium ions.

The Examiner further asserted that Hauselmann at al. teaches the method of producing an extracellular matrix, for implantation *in vivo*. The Examiner stated that Hauselmann at al. also teaches that the molar ratio of calcium ions to carboxyl groups in the gel determines the amount of cross-linking of the gel, as well as the amount of swelling (again, this is during formation of the gel, and thus is irrelevant to the issues at hand) and thus size of the gel.

The Examiner stated that Cao et al. teach the method of making and using biodegradable calcium alginate gels with osteoblasts *in vitro* for the implantation *in vivo* to generate bone growth. He also stated that the osteoblasts were suspended in 1% sodium alginate, then 0.2g of calcium sulfate was added to each milliliter of the mixture to initiate gel formation. Then, the Examiner stated that the mixture was injected into nude mice, which resulted in the new bone formation in the transplanted animals.

In conclusion, the Examiner stated that it would have been obvious to the skilled artisan to modify the teaching of Draget and Martinsen by introducing cells (osteoblasts) as taught by Hauselmann and Cao to the calcium-alginate hydrogels' composition. The Examiner further stated that one would have been motivated to utilize the gel as a scaffold for cell growth and differentiation for tissue engineering.

Further, the Examiner asserted that it would have been obvious in view of Martinsen to control the hydrogel shrinkage or swelling by transferring the hydrogels into the solutions that contain different concentration of calcium ions. He states that one would have been motivated to alter the calcium ion concentrations and the ratio of calcium ions to alginate carboxyl groups (again, irrelevant as this ratio corresponds to formation of the gel) in order to control the amount of gel swelling and shrinkage because these characteristics are highly desirable in tissues for different applications. The Examiner further asserted that the Appellant's invention pertaining to specific ion concentrations and molar ratios that results in hydrogel swelling and shrinking are the result of effective variables, which could have been readily determined in view of Draget and Martinsen.

In response to Appellant's arguments in the Amendment filed on August 28, 2003, the Examiner defended his assertions by stating that the Appellant failed to consider the combined teaching of the references, and that the combination and modification of the teachings of the prior art clearly suggested the claimed invention.

ISSUE 1. **Whether claims 1-20, 22-23, 25-38, and 40-55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, in view of Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in further view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest the selective size control of a three-dimensional hydrogel system by varying cation concentration of a separate medium into which it is introduced?**

Appellant's answer: yes

Examiner's answer: no

As highlighted above, the Examiner contends that these references, in combination, teach or suggest control of gel shrinkage and swelling by optimizing calcium ion and sodium ion concentration. **However, contrary to the Examiner's statement, none of the cited references, either alone or in combination, teach or suggest the selective size control of a three dimensional hydrogel system by varying cation concentration of a separate medium into which it is introduced.**

Generally, the references teach the following:

1) Draget et al. teaches that cation concentration in the mixture which forms hydrogels is a parameter to consider during such hydrogel formation;
2) Martinsen teaches that after binding sites at the surface of a gel bead are full, further physical changes in the gel system are small;
3) Hauselmann teaches that mechanical boundary layers may be used to control a hydrogel system; and
4) Cao teaches that a calcium source may be used to form crosslinks during formation of alginate gels.

The Appellant points out that Draget is silent as to transferring a hydrogel into a separate medium. As such, the Examiner's assertion that Draget teaches that variation in calcium ion concentration results in the *formation* of hydrogels with distinct characteristics is off point and is irrelevant to the Appellant's invention. Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites that shrinking, swelling or maintaining of the hydrogel is selectively controlled by varying a calcium ion concentration of a **separate medium** into which the hydrogel is introduced. (emphasis added) According to the court in *In re Napier*, 55 F.3d 610, 613 (Fed. Cir. 1995), "[o]bviousness cannot be established by combining the teachings of the prior art to produce

the claimed invention, absent some teaching, suggestion, or incentive supporting the combination." While Draget does teach a method for forming a hydrogel, he does not mention, teach, suggest, or provide any incentive to transfer that hydrogel into a **separate** medium.

In sharp contrast, Appellant's invention as defined in claims 1, 23 and 52 recites "... a separate medium into which hydrogel system is introduced," while Appellant's claim 11 recites "...a separate medium into which the crosslinked hydrogel system is introduced," and Appellant's claim 34 recites "...a separate culture medium into which the hydrogel system is introduced."

Appellant further points out that the Examiner's mention of the molds Draget used to form the hydrogel as determining gel dimensions is irrelevant because Appellant's invention as defined in claims 11, 23 and 52 recites selective control of "a three-dimensional hydrogel system," the gel having already been formed.

Appellant respectfully submits that Martinsen is an example of a traditional means of creating alginate gel beads. 1) Beads are the only structure which can be formed by Martinsen's method; this is in sharp contrast to Appellant's inventive hydrogel as defined in the pending claims, which may take **any** three dimensional shape. 2) The Martinsen beads are formed by allowing droplets of sodium alginate solution to fall into an aqueous solution of $CaCl_2$; this is in sharp contrast to Appellant's methods as defined in claims 1, 11, 23 and 52. Appellant's hydrogel is **not** formed in a calcium **solution**, rather the Appellant's hydrogel as defined in claim 1, 11, 23 and 52 is formed by mixing an alginate salt and a source of calcium ions and then adding a calcium releasing compound to the mixture. 3) The crosslinking density of the Martinsen-formed beads is NOT uniform– the surface is highly crosslinked, and the interior has a low (if any) crosslinking density (this is known in the art); this is also in sharp contrast to Appellant's inventive hydrogel as defined in the pending claims, which is uniformly crosslinked and is structurally homogeneous. Since Appellant's hydrogels are not formed in calcium solutions, the inventive hydrogels do not have a layer (shell) of surface crosslinking, and are therefore open pored, thus calcium ions can move in and out of the hydrogel.

Further, in Figs. 9 and 10 of Martinsen, the authors were studying how calcium competes with sodium, and how stable the gel beads were. (Figs. 7 and 8 of Martinsen are irrelevant to Appellant's recitation of selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a

medium into which the hydrogel system is introduced–Figs. 7 and 8 speak to the calcium chloride concentration of the solution used for gelation.) This is very different from Appellant's selective control of the size of the hydrogel/hydrogel system. Martinsen at p. 89, Col. 1, states in part that "when calcium ions saturate the binding sites despite the competition from sodium ions (Fig. 10), and after binding has proceeded to a maximum on the time scale (Fig. 12), further physical changes are small in the gel system. (emphasis added)

Appellant submits that Martinsen is NOT describing or suggesting selective control of the size of a hydrogel. In fact, as quoted above, once all the Martinsen binding sites (at the surface) are full, "further physical changes are small in the gel system." Martinsen seems to suggest that once binding sites are full, calcium ions may NOT move out of the system.

However, in sharp contrast, Appellant can selectively cause the inventive hydrogel to swell, shrink or maintain its size by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced.

In summary regarding Martinsen: a) the composition and structure of the Martinsen hydrogel beads is quite different from the three dimensional, uniformly crosslinked and structurally homogeneous hydrogel system of Appellant's invention as defined in the claims; b) Martinsen's aim in Figs. 9 and 10 was to study competition between sodium and calcium ions; and c) Martinsen admits that after surface binding sites are full, further physical changes are small.

Due to the disparity between Martinsen's hydrogel beads and Draget's hydrogel, the skilled artisan would **not** have been taught and/or led to believe by Martinsen that if he placed Draget's hydrogel in Martinsen's 0.9% NaCl solution, that he would be able to selectively control the shrinking, swelling or maintaining of Draget's hydrogel by varying the concentration of $CaCl_2$ in the NaCl solution. In fact, Martinsen was published in 1989, and researchers in tissue engineering have been searching for a means of controlling the size of a formed hydrogel; however, prior to Appellant's invention as defined in the pending claims, the efforts had been unsuccessful. "Failure by others to satisfy a long-felt need or develop a commercially successful product is evidence of unobviousness." *Dow Chem. Co. v. American Cyanamid Co.*, 816 F.2d 617, 623 (Fed. Cir. 1987). This would lead one to believe that Martinsen did NOT teach that which the Examiner is asserting the publication taught.

In furthering the argument regarding the failure by others to satisfy a long-felt need, a brief discussion of the Cao reference is relevant. Cao used calcium sulfate as a source of calcium to form crosslinks in alginate gels. Cao implanted his mixture *in vivo* without characterizing the structural homogeneity or mechanical properties. Although the Draget reference had published before his work, neither Cao (an expert in the field of tissue engineering), nor anyone else in the field, has derived a combination of the tissue engineering approach with Draget's gelation thus far.

Hauselmann et al. ('343) controls the hydrogel system by a **mechanical** means (the boundary layers). The passages referred to by the Examiner (Col. 7, lines 29 et seq.) speaks of molar ratio of calcium ions to carboxyl groups in the gel to determine the amount of **crosslinking** during the formation of the gel. In sharp contrast, the Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites selective control of the size of the hydrogel system by varying cation/calcium ion concentration in a separate medium into which the hydrogel is introduced.

Further, it is respectfully submitted that the Examiner, in stating that a combination of Draget with Martinsen teaches volume control of a non-bead, uniformly crosslinked three-dimensional hydrogel has used impermissible hindsight from Appellant's disclosure in order to **assume** that the Martinsen reference teaches or even suggests that **different** hydrogel structures, formed by **different** methods will react the **same** in calcium ion solutions. It is further submitted that, in doing so, the Examiner has made significant errors in the technical field at hand.

Assuming *arguendo* that the Examiner's assertions regarding Martinsen and Draget are correct, the results of such combination, in addition to the combination itself, should be obvious. As taught in Martinsen's Figure 9, the hydrogel bead swelled at calcium ion concentrations of 0.0030 M. In sharp contrast, the Appellant's hydrogel system in the medium remained **the same size** at calcium ion concentrations of 0.0020 M – 0.0030 M. Further, Martinsen teaches that the hydrogel bead shrank at calcium ion concentrations of 0.03 M and 0.05 M. Again, in sharp contrast, Appellant's hydrogel system in the medium shrank at calcium ion concentrations of **0.0040 M (an order of magnitude different from Martinsen)**.

Comparing the numbers at which Appellant's hydrogel system shrank, maintained and swelled with those shown by Martinsen, the results obtained by the

Appellant are not obvious in view of Draget and Martinsen, rather, Appellant's results are **unexpected**.

The Federal Circuit has spoken to the issue of impermissible hindsight on numerous occasions. In *In re David H. Fine*, 837 F.2d 1071, 1073-74 (Fed. Cir. 1988), the court stated:

> To reach a proper conclusion under § 103, the decisionmaker must step backward in time and into the shoes worn by [a person having ordinary skill in the art] when the invention was unknown and just before it was made. In light of *all* the evidence, the decisionmaker must then determine whether . . . the claimed invention as a whole would have been obvious at *that* time to *that* person. 35 U.S.C. § 103. The answer to that question partakes more of a nature of law than of fact, for it is an ultimate conclusion based on a foundation formed of all the probative facts. (emphasis in original) *Id.* at 1073-74, quoting *Panduit Corp. v. Dennison Mfg. Co.*, 810 F.2d 1561, 1566 (Fed. Cir. 1987).

The assumption made by the Examiner regarding **different** hydrogel structures, formed by **different** methods reacting the **same** in calcium ion solutions is not buttressed by any evidence (in fact, it is rebutted by evidence stated above from Figure 9 of Martinsen). This assertion not only seems to be gleaned from information within the Appellant's disclosure, it also flies in the face of the oft-cited maxim that chemistry is an "unpredictable" art. Further, Appellant points out that the calcium ion concentrations at which his hydrogel system in the medium shrinks, maintains size, and swells are very different from those results given by Martinsen. This difference in results lends credence to Appellant's argument that **different** hydrogel structures, formed by **different** methods, will react **differently** in calcium ion solutions (contrary to the assertion of the Examiner).

Further, the Examiner argued that as long as he takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and as long as he does not include knowledge gleaned only from the Appellant's disclosure, a reconstruction is proper. However, the Examiner "cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to depreciate the claimed invention." *Id.* at 1075. Appellant respectfully submits that the Examiner included knowledge gleaned from the Appellant's disclosure upon making his rejections. Appellant bases this submission upon the evidence herein that indicates that none of the cited references, either alone or in combination, teach or suggest the selective size control of a hydrogel system by varying cation concentration of a separate medium to which the system is introduced.

The Examiner might possibly determine that the skilled artisan might find selectively controlling the skrinking or swelling of a hydrogel system by varying calcium ion concentration of a separate medium into which the hydrogel is introduced "**obvious to try**" in view of the cited references. However, in *In re Fine*, 837 F.2d at 1075, the court stated:

> Because neither Warnick nor Eads, alone or in combination, suggests the claimed invention, the Board erred in affirming the Examiner's conclusion that it would have been obvious to substitute the Warnick nitric acid detector for the Eads sulfur dioxide detector in the Eads system. *ACS Hosp. Sys.*, 732 F.2d at 1575-77. The Eads and Warnick references disclose, at most, that one skilled in the art might find it obvious to try the claimed invention. But whether a particular combination might be "obvious to try" is not a legitimate test of patentability. [references omitted] (emphasis added).

At best, the Draget and Martinsen references in view of the '343 patent and Cao et al. show some components in bits and pieces of Appellant's inventive method as defined in the claims. The relevant art recognizes many components and concepts within its domain. Upon close investigation and scrutiny of the diverse practices in this art and its peripheral technical fields of endeavor, a fact finder is inevitably led to the conclusion that artisans can and could produce a myriad of methods and devices of apparently endless diversity from components and concepts already individually recognized as belonging to the prior art. Such speculation must not cloud the standards for evaluation of patentability over the prior art under 35 U.S.C. § 103. Properly focused, the issue centers on what would have been obvious to one of ordinary skill in the art at the time of the invention.

Obviousness is tested by what the combined references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 425 (CCPA 1981). Approaches to obviousness determinations which focus merely on identifying and tabulating missing elements in hindsight retrospect imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge (ie. the selective size control of a three dimensional hydrogel system by varying cation concentration of a separate medium into which it is introduced), and fall victim to the insidious effect of the hindsight syndrome wherein that which only the inventor taught is used against its teacher. (emphasis added). *W.L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1553 (Fed. Cir. 1983).

For all of the above reasons, it is submitted that Appellant's invention as defined in claims 1-20, 22-23, 25-38, and 40-55 is not anticipated, taught, or rendered obvious by Draget et al. in view of Martinsen et al., and further in view of Hauselmann et al. ('343) and Cao et al., either alone or in combination, and patentably defines over the art of record.

As such, it is submitted that the cited references neither teach nor suggest selective control of a hydrogel system by varying cation concentration of a separate medium into which the hydrogel is introduced as defined in Appellant's claims 1, 11, 23, 34 and 52.

ISSUE 2. **Whether claims 2, 11-20, 33, 34, 41-45 and 55 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the cited references teach or suggest culturing the crosslinked hydrogel in a medium to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro*?**

Appellant's answer: yes

Examiner's answer: no

It is further submitted that the arguments for patentability of the claims hereinabove and hereinbelow are intended to be cumulative.

As highlighted above, the Examiner contends that it would have been obvious to modify the teachings of Draget and Martinsen by introducing the cells (as taught by Hauselmann and Cao) into a calcium-alginate hydrogel composition. However, as argued above, none of the references teach or suggest culturing the hydrogel in a **separate medium** to provide a hydrogel/cell system for growing cells *in vitro*.

Cao used $CaSO_4$ as a calcium source to form crosslinks in alginate gels. The method of Cao is presented in the Appellant's specification as a control method, which results in poor gel formation (irregular shape and heterogeneity) and inferior mechanical properties. Cao implanted his mixture *in vivo* without characterizing the structural homogeneity or mechanical properties. As such, Cao does not teach or suggest any medium at all.

In sharp contrast, Appellant has found that the mechanical properties of the hydrogel maybe controlled by varying the calcium concentration of a separate medium into which the hydrogel is introduced. Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 further recites that the inventive hydrogel system may be used for growing cells when the hydrogel system in the medium is cultured.

Hauselmann discusses the use of alginate as a suitable matrix to contain and support dispersed cells, but does not mention transfer into a medium. In sharp contrast, the Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 recites culturing the three-dimensional hydrogel system in a **separate medium** for supporting and growing cells.

As discussed in reference to Issue 1, Draget neither teaches nor suggests introducing his hydrogel into any separate medium, let alone a separate medium that is adapted to stabilize the hydrogel to support cell viability.

The Examiner points out that Martinsen states, "...the high gel strength, low shrinkage, high stability towards sodium ions, and high permeability of alginates with high content of guluronic acid will normally be most advantageous for immobilization of living cells." The Appellant fails to see the relevance of this passage to his disclosed invention. Martinsen briefly mentions what "will normally be most advantageous for immobilization of living cells," but fails to teach or suggest a homogeneous crosslinked hydrogel system capable of being selectively controlled by variation of cation concentration of a medium into which the hydrogel is introduced. Martinsen does not disclose a medium at all, but rather simply a $NaCl/CaCl_2$ solution to study competition between sodium and calcium cations.

Due to the disparity between Martinsen's hydrogel beads and Draget's hydrogel, the skilled artisan would **not** have been taught and/or led to believe by Martinsen that if he placed Draget's hydrogel in Martinsen's 0.9% NaCl solution, that he would be able to selectively control the shrinking, swelling or maintaining of Draget's hydrogel by varying the concentration of the $CaCl_2$, as well as maintaining living cells in a medium.

In sharp contrast, Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 recites introduction of cells supported in a medium. Appellant's three dimensional gel structure with incorporated cells can be maintained in an *in vitro* tissue culture environment by adjusting calcium ion concentration in the culture medium.

In *In re David H. Fine*, 837 F.2d at 1073-74, the Federal Circuit stated:

> To reach a proper conclusion under § 103, the decisionmaker must step backward in time and into the shoes worn by [a person having ordinary skill in the art] when the invention was unknown and just before it was made. In light of *all* the evidence, the decisionmaker must then determine whether . . . the claimed invention as a whole would have been obvious at *that* time to *that* person. 35 U.S.C. § 103.

Appellant again submits that the Examiner has engaged in impermissible hindsight in combining the cells of Cao and Hauselmann with the hydrogels of Draget and Martinsen. AS pointed out above, none of the references teach or suggest placing hydrogels in a medium, much less a culture medium as defined in claims 2, 11, 33, 34, 45 and 55. Just as the combination of Draget and Martinsen would NOT have been obvious to one skilled in the art at the time the Appellant's invention was made, neither would a combination of Cao's cells with Draget's homogeneous gelation have been obvious. And even assuming *arguendo* such a combination were made, it would not render selective control of a hydrogel in a medium.

In sharp contrast, Appellant's invention as defined in claims 2, 11, 33, 34, 45 and 55 recites that the selectively controlled hydrogel system may be cultured in a medium for growing cells. Although the Draget reference had published before his work, neither Cao (an expert in the field of tissue engineering), nor anyone else in the field, has derived a combination of the tissue engineering approach with Draget's homogeneous gelation. This failure to derive such a combination, in addition to the lack of teaching or suggestion by the cited references, indicates that it would NOT "have been obvious at *that* time to *that* person" to prepare and form a "structurally homogeneous and mechanically strong alginate gel with defined dimensions, which can be used to incorporate living cells."

For all of the above reasons, it is submitted that Appellant's invention as defined in claims 2, 11-20, 33, 34, 41-45 and 55 is not anticipated, taught, or rendered obvious by Draget et al. in view of Martinsen et al., and further in view of Hauselmann et al. ('343) and Cao et al., either alone or in combination, and patentably defines over the art of record.

ISSUE 3: Whether claims 48 and 49 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references

teach or suggest a selectively controlled three-dimensional crosslinked hydrogel with a structurally homogeneous composition?

Appellant's answer: yes

Examiner's answer: no

It is further submitted that the arguments for patentability of the claims hereinabove and hereinbelow are intended to be cumulative.

As previously stated, the Examiner argues that it would have been obvious in view of Martinsen to control the hydrogel shrinkage or swelling by transferring the hydrogels into solutions that contain different concentrations of calcium ions. Further, the Examiner argues that it would have been obvious to combine Draget's hydrogel with Martinsen's alleged teaching.

Draget does mention a "homogeneous gel." However, as explained in reference to Issue 1, Draget fails to teach or suggest the introduction of the hydrogel into a separate medium. Therefore, Appellant submits that by combining the Draget homogeneous hydrogel with the alleged teaching of Martinsen, the Examiner is concluding that the homogeneous hydrogel of Draget will behave the same as the non-homogeneous, highly crosslinked surface hydrogel bead of Martinsen.

It is respectfully submitted that the Examiner, in stating that Martinsen teaches volume control of a non-bead, uniformly crosslinked hydrogel has used impermissible hindsight from Appellant's disclosure in order to **assume** that the Martinsen reference teaches or even suggests that **different** hydrogel structures, formed by **different** methods will react the **same** in calcium ion solutions. It is further submitted that, in doing so, the Examiner has made errors in the technical field at hand.

The assumption made by the Examiner regarding **different** hydrogel structures (homogeneous versus highly crosslinked surfaces with low to no crosslinked interior), formed by **different** methods reacting the **same** in calcium ion solutions is not buttressed by any evidence. In fact, as shown above, the Examiner's assertion is rebutted by teachings of Martinsen (Figure 9). The Examiner's assertion not only seems to be gleaned from information within the Appellant's disclosure, it also flies in the face of the oft-cited maxim that chemistry is an "unpredictable" art.

For all of the above reasons, it is submitted that Appellant's invention as defined in claims 48 and 49 is not anticipated, taught, or rendered obvious by Draget et al.

in view of Martinsen et al., and further in view of Hauselmann et al. ('343) and Cao et al., either alone or in combination, and patentably defines over the art of record.

ISSUE 4: Whether claims 22, 32, 40, 46, 47 and 52 are patentable over Draget et al., Carb. Poly. 14:159-178, 1991, Martinsen et al., Biotech. Bioeng. 33:79-89, 1989, in view of Hauselmann et al., U.S. Patent No. 5,658,343 ('343) and Cao et al., Book of Abstracts, BIOT-212, 211th ACS National Meeting, New Orleans, 1996, under 35 U.S.C. § 103(a) when none of the references teaches swelling of a three dimensional hydrogel at 0.0005M – 0.001M, shrinking at 0.004M and maintaining at 0.002M – 0.003M?

Appellant's answer: Yes

Examiner's answer: No

It is further submitted that the arguments for patentability of the claims hereinabove are intended to be cumulative.

The Examiner asserts that the combination of the teachings of Draget and Martinsen is obvious and that the combination in further view of Hauselmann and Cao results in the Appellant's claimed invention. However, Appellant points out that if such a combination is obvious, the results of such combination should also be obvious. In *In re Payne*, 606 F.2d 303, 313 (C.C.P.A. 1979), the court stated, "[a]n obviousness rejection based on similarity in chemical structure and function entails the motivation of one skilled in the art to make a claimed compound, in the expectation that compounds similar in structure will have similar properties." In sharp contrast, the hydrogel of Draget is NOT similar in structure to the hydrogel bead of Martinsen. Further, Appellant submits that even assuming *arguendo* that the skilled artisan combined Draget and Martinsen as suggested by the Examiner, the results of such combination are not obvious.

In Figure 9 of Martinsen, the hydrogel bead swelled at a calcium ion concentration of 0.0030 M. In sharp contrast, the Appellant's hydrogel system in the medium remained the same size at calcium ion concentrations ranging between 0.0020 M – 0.0030 M.

Further, Martinsen teaches that the hydrogel bead shrank at calcium ion concentrations of 0.03 M and 0.05 M. Again, in sharp contrast, Appellant's hydrogel system in the medium shrank at calcium ion concentrations of 0.0040 M. The shrinking of

the Appellant's hydrogel resulted at a calcium ion concentration which is an order of magnitude less than that shown in Martinsen.

Still further, Martinsen teaches the swelling of the hydrogel bead at 0.001 M, 0.003 M and at 0.005 M, the swelling trend generally moving from 0.001M upward. Appellant showed swelling at calcium ion concentrations ranging between about 0.0005 M and about 0.001 M, the swelling trend generally moving from 0.001M downward.

Appellant's results, which are unexpectedly different than those taught by Martinsen, further support the Appellant's assertion that **different** hydrogel structures, formed by **different** methods, will react **differently** in calcium ion solutions (contrary to the assertion of the Examiner).

Appellant respectfully submits that none of the references cited by the Examiner teach or suggest "compounds similar in structure" that "have similar properties" as those of the Appellant's invention as defined in claims 22, 32, 40, 46, 47 and 52.

In summary,

1) Draget et al. teaches that cation concentration in the mixture which forms hydrogels is a parameter to consider during such hydrogel formation. Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites the introduction of an already formed hydrogel into a **separate** medium and selectively controlling shrinking, swelling, and maintaining by varying the cation concentration of a separate medium into which the hydrogel is introduced.
2) Martinsen teaches the formation of gel beads only by allowing droplets of sodium alginate solution to fall into an aqueous solution of $CaCl_2$. Appellant's hydrogel as defined in claim 1, 11, 23, 34 and 52 is **not** formed in a calcium **solution** and can take on any three-dimensional shape.
3) Martinsen teaches a hydrogel with a highly crosslinked surface. After binding sites at the surface of the gel bead are full, further physical changes in the gel system are small. Appellant's inventive hydrogels as defined in claim 1, 11, 23, 34 and 52 do not have a layer (shell) of surface crosslinking, and are therefore open pored, thus calcium ions can move in and out of the hydrogel, allowing selective shrinking, swelling, or maintaining of its size.
4) Hauselmann teaches that mechanical boundary layers may be used to control a hydrogel system. Appellant's invention as defined in claims 1, 11, 23, 34 and 52

recites that selective control of shrinking, swelling, or maintaining the hydrogel is accomplished by varying the calcium ion concentration of a separate medium into which the hydrogel is introduced (no mechanical boundaries).

5) Cao teaches that a calcium source may be used to form crosslinks during formation of alginate gels for direct implantation *in vivo*. Appellant's invention as defined in claims 1, 11, 23, 34 and 52 recites culturing the hydrogel in a medium to provide a hydrogel/cell system for growing cells *in vitro*.

CONCLUSION

For the reasons stated above, it is submitted that there is no teaching or suggestion in any of the cited references to selectively control the size of a three-dimensional hydrogel system by varying cation concentration of a separate medium into which the hydrogel is introduced; nor do the cited references teach or suggest culturing the crosslinked hydrogel in a medium to provide a three-dimensional crosslinked hydrogel/cell system for growing cells *in vitro*; still further, the cited references do not teach or suggest selective control of a hydrogel with a structurally homogeneous composition; yet further, none of the cited references teach or suggest shrinking, swelling or maintaining of a hydrogel with calcium ion concentration molarities as defined in Appellant's claims.

Thus, it is respectfully submitted that Appellant's invention as set forth in claims 1-20, 22-23, 25-38, and 40-55 patentably defines over the cited references and is not anticipated, taught or rendered obvious thereby.

As such, it is respectfully submitted that the Examiner's final rejection of claims 1-20, 22-23, 25-38, and 40-55 is erroneously based, and its reversal is respectfully requested.

No oral hearing is requested.

Appellant's Attorney's Deposit Account may be charged in the amount of $165.00 to cover the Appeal Brief filing fee.

This Appeal Brief is being filed in triplicate, including one original and two copies.

Respectfully submitted,

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APPENDIX
CLAIMS AT ISSUE IN APPEAL OF S.N. 09/255,963

1. A method comprising the steps of:

mixing an alginate salt and a source of calcium ions to provide a mixture;

adding a calcium releasing compound to the mixture to provide a three-dimensional crosslinked hydrogel system; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced.

2. The method of Claim 1, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells in vitro.

3. The method of Claim 1, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

4. The method of Claim 1, wherein the alginate salt is prepared from an alginate source selected from Macrocystis pyrifera and Laminaria hyperborea.

5. The method of Claim 1, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

6. The method of Claim 1, wherein the calcium releasing compound is D-glucono-δ-lactone.

7. The method of Claim 1, wherein the source of calcium ions is calcium carbonate and the calcium releasing compound is D-glucono-δ-lactone, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

8. The method of Claim 1, further comprising the step of implanting the three-dimensional crosslinked hydrogel system.

9. The method of Claim 1, wherein the three-dimensional crosslinked hydrogel system has a thickness of between about 4 mm and about 8 mm, and a diameter of approximately 18 mm.

10. The method of Claim 1, wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio of 0.27.

11. A method for tissue engineering *in vitro*, the method comprising the steps of:

mixing cells, an alginate salt and a source of calcium ions to provide a mixture;

adding a calcium releasing compound to the mixture to provide a crosslinked hydrogel;

selectively controlling shrinking, swelling or maintaining of the crosslinked hydrogel by varying a calcium ion concentration of a separate medium into which the crosslinked hydrogel is introduced; and

culturing the crosslinked hydrogel in the medium to provide a three-dimensional crosslinked hydrogel/cell system for growing the cells *in vitro*.

12. The method of Claim 11, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

13. The method of Claim 11, wherein the alginate salt is prepared from an alginate source selected from Macrocystis pyrifera and Laminaria hyperborea.

14. The method of Claim 11, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

15. The method of Claim 11, wherein the calcium releasing compound is D-glucono-δ-lactone.

16. The method of Claim 11, wherein the source of calcium ions is calcium carbonate and the calcium releasing compound is D-glucono-δ-lactone, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

17. The method of Claim 11, further comprising the step of implanting the three-dimensional crosslinked hydrogel/cell system.

18. The method of Claim 11, wherein the three-dimensional crosslinked hydrogel/cell system has a thickness of between about 4 mm and about 8 mm, and a diameter of approximately 18 mm.

19. The method of Claim 11, wherein the three-dimensional crosslinked hydrogel/cell system has a calcium ion to carboxyl molar ratio of 0.27.

20. The method of Claim 11, wherein the cells are osteoblasts.

22. The method as defined in claim 1 wherein the hydrogel system swelled at calcium ion concentrations in the medium between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration in the medium of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations in the medium between about 0.0020 M and about 0.0030 M.

23. A method for preparing a three-dimensional hydrogel system, the method comprising the steps of:

adding a calcium-releasing compound to a mixture of at least one hydrophilic polymer comprising an alginate salt and a source of calcium cations to provide a three-dimensional crosslinked hydrogel system; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced.

25. The method as defined in claim 23 wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate.

26. The method as defined in claim 23, wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate.

27. The method as defined in claim 26 wherein the calcium releasing compound is D-glucono-δ-lactone.

28. The method as defined in claim 27 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

29. The method as defined in claim 23 wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

30. The method as defined in claim 29 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

32. The method as defined in claim 23 wherein the hydrogel system swelled at calcium ion concentrations between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations between about 0.0020 M and about 0.0030 M.

33. The method as defined in claim 23, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells in vitro.

34. A three-dimensional crosslinked hydrogel composition, consisting essentially of:

at least one hydrophilic polymer comprising an alginate salt;

a source of calcium cations;

a calcium-releasing compound, whereby a mixture of the at least one hydrophilic polymer, the source of calcium cations and the calcium-releasing compound forms the crosslinked hydrogel composition; and

a separate culture medium into which the hydrogel composition is introduced, the culture medium having a predetermined calcium ion concentration, wherein the predetermined calcium ion concentration determines the shrinking, swelling or maintaining of the crosslinked hydrogel composition.

35. The composition as defined in claim 34, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate; wherein the source of calcium cations is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate; and wherein the calcium-releasing compound is D-glucono-δ-lactone.

36. The composition as defined in claim 35 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

37. The composition as defined in claim 35 wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

38. The composition as defined in claim 37 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

40. The composition as defined in claim 34 wherein when the predetermined calcium ion concentration is between about 0.0020 M and about 0.0030 M, the hydrogel composition remains substantially the same size.

41. The composition as defined in claim 45 wherein the cells are at least one of osteoblasts and cells which secrete a medically useful compound.

42. The method of claim 2 wherein the cells secrete a medically useful compound.

43. The method of claim 11 wherein the cells secrete a medically useful compound.

44. The method of claim 33 wherein the cells are at least one of osteoblasts and cells which secrete a medically useful compound.

45. The three-dimensional crosslinked hydrogel composition as defined in claim 34, further comprising cells incorporated into the hydrogel composition, thereby forming a hydrogel/cell system.

46. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein when the predetermined calcium ion concentration is between about 0.0005 M and about 0.0010 M, the hydrogel composition swelled.

47. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein when the predetermined calcium ion concentration is about 0.0040 M, the hydrogel composition shrank.

48. The method as defined in claim 1 wherein the three-dimensional crosslinked hydrogel system is structurally homogeneous.

49. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein the composition is structurally homogeneous.

50. The method as defined in claim 1 wherein the source of calcium ions is in powder form.

51. The three-dimensional crosslinked hydrogel composition as defined in claim 34 wherein the source of calcium cations is in powder form.

52. A method for preparing a three-dimensional hydrogel system, the method comprising the steps of:

adding a calcium-releasing compound to a mixture of at least one hydrophilic polymer comprising an alginate salt and a source of calcium cations to provide a three-dimensional crosslinked hydrogel system, wherein the calcium releasing compound is D-glucono-δ-lactone, wherein the alginate salt is selected from the group consisting of sodium alginate and potassium alginate, and wherein the source of calcium ions is selected from the group consisting of calcium carbonate, calcium sulfate, and calcium sulfate dihydrate; and

selectively controlling shrinking, swelling or maintaining of the hydrogel system by varying a calcium ion concentration of a separate medium into which the hydrogel system is introduced, wherein the hydrogel system swelled at calcium ion concentrations between about 0.0005 M and about 0.0010 M; wherein the hydrogel system shrank at a calcium ion concentration of about 0.0040 M; and wherein the hydrogel system remained substantially the same size at calcium ion concentrations between about 0.0020 M and about 0.0030 M;

wherein the three-dimensional crosslinked hydrogel system has a calcium ion to carboxyl molar ratio ranging between about 0.09 and about 0.9.

53. The method as defined in claim 52 wherein the source of calcium ions is calcium carbonate, and wherein the molar ratio of the calcium carbonate to the D-glucono-δ-lactone is 0.5.

54. The method as defined in claim 53 wherein the calcium ion to carboxyl molar ratio ranges between about 0.18 and about 0.72.

55. The method as defined in claim 54, further comprising the step of culturing the three-dimensional crosslinked hydrogel system in the medium for growing cells *in vitro*.